

Integrating research and restoration: the establishment of a long-term woodland experiment in south-eastern Australia

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ABSTRACT

Long-term studies of ecological restoration, within a designed randomized experimental framework, are uncommon; however, such projects provide hitherto under-utilized opportunities to inform both evidence-based management planning and action, and ecological theory. Baseline data collected prior to the application of treatments allows accurate estimation of changes taking place on the experimental units, and random allocation of treatments ensures that relations between causes and effects can be established. This is critical to effective active adaptive management. In this paper, we outline the establishment phase of a new long-term ecological restoration experiment in south-eastern Australia, that will test ways of improving critically endangered box gum grassy woodlands for biodiversity.

In the experimental design, treatments include the addition of 2000 tonnes of coarse woody debris, exclusion of kangaroos and fire. Random variation in biophysical variables occurs at several levels. To facilitate accurate estimation of key main effects, selected high order interactions are partially confounded with 'random' block terms. Response variables include: plants, birds, small mammals, reptiles and invertebrates. Analysis of baseline data across selected response variables confirmed no pre-treatment effects.

The experiment provides a strong inferential framework for tracking the effects of restoration treatments on woodland biodiversity over coming years. It also provides a model for other similar experiments that integrate restoration and research. A newly constructed feral animal-proof fence, that will allow reintroduction of locally extinct species, including ecosystem engineers, will provide additional opportunities to research the woodland restoration process. This experiment will become a long-term ecological research site, and an 'outdoor laboratory' for ecological restoration research, and community and student learning.

Key words: box-gum grassy woodlands, *Eucalyptus blakelyi*, *Eucalyptus melliodora*, evidence-based conservation, feral animal-proof fence, Mulligans Flat – Gorooyarroo Woodland Experiment.

Introduction

Long-term ecological research is recognized globally as critical for understanding environmental change (Hobbie *et al.* 2003; Kratz *et al.* 2003; Turner *et al.* 2003). Long-term studies of ecological phenomena are essential for understanding slow processes, subtle processes, processes with high annual variability, rare events or episodic phenomena, and complex phenomena (Likens 1983; Strayer *et al.* 1986; Lindenmayer and Likens 2010), and for formulating and testing ecological theory (Franklin 1989). However, in Australia, there has been a paucity of long-term ecological studies, with few running more than 50 years and none of those having replicated plots from different experimental treatments (Lunt 2002).

Designed experiments allow the determination of causal links between manipulated factors and their measured effects on an ecosystem (Tilman 1989). This makes them particularly useful in restoration ecology where relationships between actions and outcomes can be established and then incorporated into management practices. New long-term ecological restoration experiments are now being established in Australia (e.g. Margules 1992; Lindenmayer *et al.* 2001), but they are still rare. Large-scale ecological restoration projects are under-utilized as experiments to inform both management action and ecological theory (Holl *et al.* 2003). There is also a growing recognition of the

need for conservation decisions to be evidence-based (Sutherland *et al.* 2004). Evidence-based management is also recognized as a desirable objective by government land management agencies; however, there is often a lack of resources and expertise to undertake the underpinning research. There is a critical need for long-term experiments in ecological restoration to inform conservation decision-making, particularly in highly modified or endangered ecosystem types.

An example of a highly modified endangered ecosystem in Australia is temperate woodland, which is one of the vegetation types most profoundly affected by European settlement (Yates and Hobbs 1997; Hobbs and Yates 2000). A range of human-induced disturbances have led to a drastic reduction in the extent and condition of temperate woodland ecosystems. These threatening processes include: direct vegetation clearing, vegetation modification and fragmentation, altered grazing regimes, removal of fallen timber and dead trees (often for firewood), inappropriate re-vegetation activities, physical disturbance (e.g. 'tidying up', cultivation, development), adding fertilizers, introduction of invasive exotic pest animals and plants and human-induced climate change. These have resulted in changes to soil health and nutrient cycling (including disruption of mycorrhizal fungi communities), changed levels of tree regeneration (in particular, cessation of recruitment), loss of understory vegetation, changed ground flora composition, weed invasion, declining tree health, invasive exotic pest animals, changed hydrology, decline or extinction of native fauna and elevated salinity (Yates and Hobbs 1997; Hobbs and Yates 2000; Landsberg 2000; Prober *et al.* 2002). Most of these threatening processes and their consequences are ongoing, and do not act in isolation, but interact synergistically (Yates and Hobbs 1997). This has resulted in the local, regional or global extinction of many plants and animals (Hobbs and Yates 1997; Hobbs and Yates 2000), including many small to medium-sized mammals, that played a 'keystone' role in these ecosystems (Burbidge and McKenzie 1989; Short and Smith 1994; Dickman 1994; Short 1998; Martin 2003). These losses are, in themselves, threatening processes.

The need to conserve remaining areas of native temperate woodlands is clear, and there is a general consensus about the need for remaining stands of woodland to be actively managed to maintain and improve their condition (Yates and Hobbs 1997; Hobbs and Yates 2000; Prober *et al.* 2002), to facilitate their regeneration and restoration (Spooner *et al.* 2002), and to conserve key elements of biota, such as birds and reptiles associated with native ground covers and understorey (Bennett *et al.* 2000; Freudenberger 1999). However, the effectiveness of some management interventions is poorly quantified because of a lack of high quality long-term experimental data that allows strong inference of treatment cause and effect.

In this paper, we outline a designed restoration experiment: the 'Mulligans Flat – Goorooyarroo Woodland Experiment'. The project aims to understand

ways of restoring the structure and function of temperate woodlands to increase biodiversity. A set of key ecosystem manipulations have been chosen to investigate how to reverse the decline in the biodiversity of these woodlands. These are: the addition of coarse woody debris, kangaroo exclusion and fire. The manipulations have been applied in a randomised incomplete block design which maximises the accuracy with which the effects can be estimated (as well as enabling interactions to be estimated). In addition, feral animal species have been excluded from one of the reserves (Mulligans Flat) since June 2009.

In establishing this designed restoration experiment, our intention is for it to become a long-term ecological research site and an 'outdoor laboratory' for ecological restoration research, and community and student learning. In this paper, we:

1. outline the rationale and experimental design;
2. present selected baseline data to demonstrate that no pre-treatment effects exist, and to quantify variability;
3. provide a central reference document to which researchers can refer when undertaking research at the site in the future;
4. provide a model for the establishment of similar projects that integrate ecological restoration and research in the future.

Background

Yellow Box- Blakely's Red Gum Grassy Woodlands

Yellow Box- Blakely's Red Gum Grassy Woodland (a type of temperate woodland) is an ecological community dominated by mixtures of Yellow Box *Eucalyptus melliodora* and Blakely's Red Gum *E. blakelyi* (ACT Government 2004a). In combination with White Box *E. albens*, this community once occurred over extensive areas of south-eastern Australia, including the western slopes and tablelands of the Great Dividing Range, southern Queensland, western New South Wales, the Australian Capital Territory (ACT) and Victoria (Beadle 1981; Department of Environment and Heritage 2006). Since European settlement, 92% of White Box-Yellow Box-Blakely's Red Gum Grassy Woodland has been cleared (over 5 million hectares) (Threatened Species Scientific Committee 2006) and consequently, it is recognized nationally as a critically endangered ecological community (Department of Environment and Heritage 2006).

Research site

The study area is in north-eastern ACT, and comprises two adjacent nature reserves - Mulligans Flat and Goorooyarroo Nature Reserves (see Figure 1). Mean daily temperatures for each month in the area range from a minimum of 6.5 to a maximum of 19.7° celsius, and mean annual rainfall is 615.9 mm (1939-2009; Bureau of Meteorology 2009).

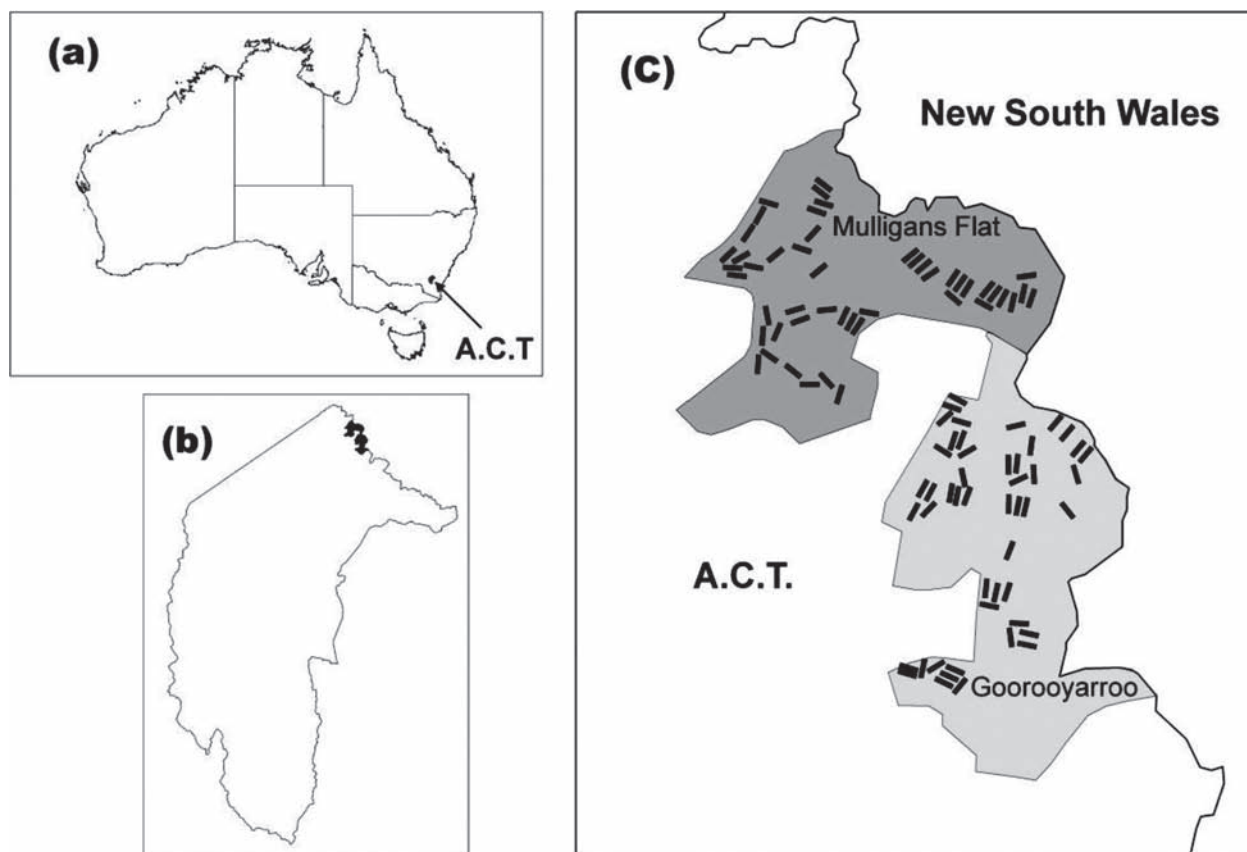


Figure 1 – The location of Mulligans Flat and Goorooyarroo Nature Reserves in the northern Australian Capital Territory (A.C.T.). (a) the A.C.T. within Australia. (b) the reserves within the A.C.T. (c) the experimental 1 hectare sites within the reserves.

A general summary of the soils in the reserves can be found in McIntyre *et al.* (2010). Together, the Mulligans Flat-Goorooyarroo Nature Reserves total 1494 ha, and contain 1210 ha of Yellow Box – Blakely's Red Gum Grassy Woodland (Felicity Grant, 2010, personal communication)- the largest and most intact example of its type in the ACT (ACT Government 2004a). Mulligans Flat was gazetted in 1994 (Sharon Lane, 2009, personal communication) after a major campaign by community groups (Lindenmayer 1992). Goorooyarroo was gazetted in 2006 (Sharon Lane, 2009, personal communication) following a reassessment by the ACT Government of its conservation priorities in the face of new residential development in Gungahlin (Canberra's newest town). As well as hill tops that have historically been protected, the reserve includes significant low lying areas – supporting Yellow Box – Red Gum Grassy Woodland and derived grasslands. These are areas that have typically been managed as rural properties and grazed by sheep and cattle before being developed as suburbs. It was calculated that \$300 million in revenue from potential land sales was foregone to establish the reserve (ACT Government 2004b).

The Mulligans Flat-Goorooyarroo Nature Reserves have several unique features that make them suited for research:

1. they support one of the most extensive remaining areas of publicly managed native woodlands dominated by critically endangered Yellow Box-Blakely's Red Gum Grassy Woodland in Australia;
2. extensive fine-scale mapping data of vegetation cover, structure and past management history is available from Government databases and records;
3. there is one long-term owner and land manager – the ACT Government;
4. there is very strong support from the ACT Government Parks management agency for this project, particularly the implementation of experimental treatments;
5. the reserves are situated near a large centre of population (Canberra), and universities and research institutions, which means they are especially suitable as an 'outdoor laboratory' for research, teaching and learning at all levels.
6. there is a well-educated local community and a number of special interest groups that actively support woodland conservation and restoration activities.

Australian National University-ACT Government Collaboration

The Mulligans Flat – Goorooyarroo Woodland Experiment is funded through an Australian Research Council Linkage Grant (LP0561817) scheme which is designed to foster genuine collaboration between Universities and 'Industry Partners'. Industry partners contribute cash and in-kind contributions to support a project. In this project, the ACT Government in-kind contributions include staff time, specialist logistical

and procurement skills, and materials. Cooperative long-term ecological research projects with government agencies, such as this, are recognized as a way to improve the viability of large-scale experiments because they own large land holdings and can provide security and stability (Franklin 1989). As the reserves constitute some of the best and largest examples of Yellow Box-Blakely's Red Gum Grassy Woodland in public ownership in south-eastern Australia, it is one of the few places where an array of management regimes (and their interactions) can be investigated in a comprehensive manner.

Materials and methods

Experimental structure

The vegetation structure and composition and the topography of the two reserves justifies the Mulligans Flat – Gorooyarroo Woodland Experiment being split into two 'companion' experiments. While both reserves will have the same treatments applied, Mulligans Flat also had a feral animal-proof fence built around it (Figure 2; ACT Government 2009) to allow the reintroduction of locally extinct species of animals. This management action is neither randomized nor replicated, so a formal statistical comparison of the presence of the feral animal-proof fence with its absence is not possible. However, the removal of feral animal species, and the reintroduction of native animals is expected to have profound effects on the ecosystem. These effects may interact with the experimental treatments. Gorooyarroo will continue to have feral animals present, and as such, results will be more typical of similar situations in the wider landscape. Comparisons will be made between the results of the two experiments where appropriate.

The multi-level experiment consists of 24 'polygons' selected randomly from a larger set of candidates, with four 1 hectare 'sites' per polygon (96 sites in total; see Figure 3). Fixed effects are factorial combinations of the treatments listed below (as well as vegetation

structure), which occur at either the polygon level or the site level. Within each site, two points or 'plots' have been established (Figure 3). The experiment provides a wide inductive basis for general inferences relating to Yellow Box-Blakely's Red Gum Grassy Woodland management regionally. The statistical framework also lends itself to additional studies on aspects of woodland and restoration management. Hence, the experiment's potential as a long term ecological research site and outdoor laboratory.



Figure 2 – The feral animal exclusion fence in Mulligans Flat Nature Reserve. Photo, A. Manning.

A polygon



A 1ha site 200m x 50m



A plot - observational unit



Figure 3 Experimental 'polygons' are homogenous areas of vegetation structure and type within which four 1 ha 'sites' were placed. Within these, depending on the phenomena being investigated, are 'plots' – an observational unit

The polygons as stratifying experimental units

The key stratifying unit of this experiment is the 'polygon' (see Figure 3). These are defined as homogenous areas of vegetation structure and type, and were surveyed, assessed and classified by ACT government staff for management purposes. From this database, four combinations of vegetation structure were derived:

1. High Tree Cover, High Shrub Cover (HTHS)
2. High Tree Cover, Low Shrub Cover (HTLS)
3. Low Tree Cover, Low Shrub Cover (LTLS)
4. Low Tree Cover, High Shrub Cover (LTHS)

All four combinations (vegetation classes) did not occur in both reserves, and some were re-classified to provide sufficient polygons (experimental classes) for random allocation. Some polygons also were split to produce two for selection when suitable polygons were unavailable. The three experimental classes used were: Mulligans Flat - HTHS, LTHS and LTLS; Goorooyarroo - HTHS, HTLS, LTLS. Experimental class has been used for the purposes of this analysis. All polygons used have been classified as Yellow Box-Blakely's Red Gum Grassy Woodland by the ACT Government. Vegetation structure is also considered as an experimental treatment (although not an active manipulation). The polygon is the experimental unit for inferences relating to vegetation structure and grazing treatments.

The site level

Within each experimental polygon, there are four 1 hectare 'sites' (200 m x 50 m; Figure 3). Each site is marked in the field along the long axis by plastic pegs at the 0 m and 200 m points, and with star pickets at the 50 m and 150 m points (see Figure 3). The fire and coarse woody debris (CWD) treatments are applied at the site level. The site is the observational unit for reptiles and for vegetation over 0.5 m height.

The plot

Each 50 m radius plot (2 per site) is centred on a star picket (Figure 3). The plot is the observational unit for birds.

Sub-plot woodland 'elements'

Within Yellow Box-Blakely's Red Gum Grassy Woodlands, there are key structural and ecological elements. Such elements can play keystone roles in the ecosystem and act as 'hotspots' of ecological function such as nutrient input and cycling. These elements include trees and coarse woody debris. To understand the effects of these elements on ecological processes and biodiversity, measurement at the sub-plot level is stratified by woodland 'element'. In the first instance, woodland elements are the observational unit for invertebrates (see below), although results can be aggregated to the site level. In the future, additional studies will take place based on observations at the woodland element level (e.g. Barton *et al.* 2009), which will allow spatial and process modeling of the woodland ecosystem and associated biodiversity.

Treatments – rationale and implementation

The treatments in the Mulligans Flat - Goorooyarroo Woodland Experiment are: addition of CWD, kangaroo exclusion and fire. Vegetation structure is a key stratifying factor. It is also treated as a fixed effect in analyses. In the following section, the rationale for their inclusion and the treatment design is outlined.

Coarse woody debris

In temperate forests globally, fallen timber, or 'coarse woody debris', has a major influence on the structure and function of ecosystems (Harmon *et al.* 1986). There is limited published data on CWD in Yellow Box – Blakely's Red Gum Grassy Woodlands (Manning *et al.* 2007; Gibbons *et al.* 2008). The effect of the broadscale removal of CWD on biodiversity and ecosystem function is recognised in Australia (Reid 1999; Driscoll *et al.* 2000). Coarse woody debris takes a long time to accumulate in woodlands and reversing the negative effects of loss cannot be achieved simply by preventing removal. To overcome the lack of CWD and its slow accumulation, deliberate augmentation can be an effective way of reversing negative effects (e.g. Michael *et al.* 2004; Mac Nally and Horrocks 2007; 2008). To our knowledge, this has never been attempted in Yellow Box – Blakely's Red Gum Grassy Woodlands on the scale undertaken in this experiment (see below).

There are no pre-European 'benchmark' sites containing Yellow Box-Blakely's Red Gum Grassy Woodland (Prober *et al.* 2002) from which to assess 'natural' levels of CWD (Manning *et al.* 2007). Therefore, the amount of CWD to be added to the treatment sites was determined from an earlier investigation in both reserves (Manning *et al.* 2007). In that study, it was found that the median CWD load was 19.3 m³ per ha (20.5 tonnes/ha). This was used as a target tonnage for two treatment types (see below). It was not assumed that this represented 'natural' levels of CWD, but rather that it would produce a significant treatment effect. As well as overall tonnage, there is some evidence that the distribution of CWD is also important to organisms (Mac Nally *et al.* 2001; Arthur *et al.* 2003). Understanding the effect of the pattern of CWD distribution is important because: (1) it is useful for managers to be able to optimise the effectiveness of CWD augmentation as a management practice; (2) because there could be different levels of effort and disturbance associated with placing CWD in the field; (3) the amount and spatial arrangement of cover from predators affects the 'useability' of a landscape by animals (Laundré *et al.* 2001; Searle *et al.* 2008). The CWD treatments were as follows:

1. No added CWD (controls);
2. 20 tonnes of CWD per ha distributed in a dispersed pattern – 'Dispersed';
3. 20 tonnes of CWD per ha distributed to mimic a natural tree fall - 'Clumped';
4. 40 tonnes of CWD per ha with both dispersed and clumped distribution sites - 'Dispersed and clumped'.

A total of 2000 tonnes of CWD was required for the experimental treatments. Sourcing and moving this quantity of CWD was a significant logistical exercise. The only source of significant quantities of coarse woody debris was from an ACT Government program of street tree renewal. It was decided that eucalypts of any species would be used. However, smaller twigs, branches and leaves were removed to avoid inadvertent introduction of seeds of species that were not locally native. Twenty tonnes of CWD was calculated to constitute approximately 31 logs, if a range of sizes was used. Logs were dispersed using a forestry forwarder for 13 days during October 2007 (Figure 4). Weather conditions were dry during this period, which helped minimise site disturbance. The forwarder operator was asked to mix log sizes and types when loading the vehicle. Substantial efforts were made to minimise disturbance to the reserves. Where possible, logs were dropped or 'flung' into the site from outside the area – so that soil compaction within the sites was minimised.

Each log was labeled with a unique ID tag for long term research. Levels of pre-treatment CWD (logs over 10 cm diameter) and 'fine woody debris' (FWD, 2 – 10 cm diameter) were assessed using the line intersect method (Warren and Olsen 1964; Van Wagner 1968) as part of the vegetation assessments for trees (see below). This will also allow analysis of the relationship between tree cover and woody debris inputs and also, in the long-term, accumulation rates.

Kangaroo exclusion

Kangaroo densities in Mulligans Flat and Goorooyarroo are very high by national standards (Howland 2008). Density estimates of 1.42 animals ha⁻¹ were made in Mulligans Flat in August/September 2008 and 1.95 animals ha⁻¹ in Goorooyarroo from October 2007 to January 2008 (Howland 2008; Howland, unpublished data). High levels of kangaroo grazing pressure have had a visible effect on the ground layer in both reserves (McIntyre *et al.* 2010). Literature on the effects of high kangaroo grazing on woodland ecosystems and biodiversity is limited (Howland 2008). However, it can reasonably be assumed that grazing reduces biomass (affecting food and shelter availability for fauna), and alters moisture retention, nutrient cycling, plant community structure and composition.

To examine the effects of kangaroo grazing, exclosures were established around half (48) of the sites to significantly reduce the density of kangaroos. Kangaroo densities inside and outside exclosures were assessed before and after kangaroos were excluded (Howland 2008; Howland, unpublished data). Assignment of exclosure treatments to polygons was random although, where possible, exclusion polygons were fenced together for logistical reasons.

Two types of fences were built: (1) by raising the height of existing fences (in Goorooyarroo; Figure 5); (2) completely new exclusion fences (in Mulligans Flat). Fences were built by ACT Government staff, Conservation Volunteers Australia (www.conservationvolunteers.com.au) and a fencing contractor. Complete exclusion of grazing can be detrimental to vegetation communities that have evolved with grazing (Gordon *et al.* 2004), therefore, the purpose

of the fences was not to fully exclude kangaroos, but rather to significantly reduce relative numbers and effects. Kangaroos were herded out of exclosures. After herding, some kangaroos remained inside the exclosures, and some returned after being herded. When numbers increased inside above 0.5 per ha – which was deemed the point at which they would have a significant effect (D. Fletcher and B. Howland, personal communication) – kangaroos were herded out again by park management staff. Kangaroo numbers are monitored with direct counts and pellet counts (Howland 2008; Howland, unpublished data).

Fire

Fire is a key form of disturbance in temperate woodlands (Hobbs 2002; Prober *et al.* 2004). However, its effects are complex, and vary with the woodland system in question, the components of fire regimes (*sensu* Gill *et al.* 1981) and other factors. Fires can assist nutrient recycling, promote regeneration of some plant species, reduce the dominance of others such as dense thatches of *Themeda australis* (allowing other plants to grow), and contribute to the maintenance



Figure 4 – Placement of logs on an experimental site with forestry forwarder. Photo, A. Manning.



Figure 5 - A kangaroo exclusion fence in Goorooyarroo Nature Reserve. Photo, A. Manning.

of species richness in the ground and understorey layers (Prober *et al.* 2002). Limited research has been conducted on fire in this ecosystem type (Hobbs 2002). For these reasons, fire was included as a treatment to examine direct effects on plants (e.g. structure, composition, biomass) and animals, and the interacting effects with other treatments. Fire will be applied to half (48) of the sites. Pre-1750 fire regimes in Yellow Box-Blakely's Red Gum Grassy Woodlands are essentially unknown (Threatened Species Scientific Committee 2006). Therefore, the intention is not to try to recreate past fire regimes, but rather to look at the ecological effect of fire. This question is of particular importance to the management of nature reserves in the ACT because of a requirement to reduce fire fuel hazard along the urban edge for asset protection, and need for a better understanding of ecological effects of this management action. At time of publication, fire treatments have been delayed due to the combined effects of drought and intense kangaroo grazing on biomass levels (see Results).

Vegetation

Trees and shrubs are the dominant structural element in the woodland ecosystem, and the stratifying variable for this experiment. Consequently, they are included as treatments in statistical analyses.

Treatment interactions

There may be important interactions between the treatments outlined above. For example, fallen logs can act as mesic refuges and micro-fire-breaks for biota (reviewed in Lindenmayer *et al.* 2002). They also can provide fuel that increases fire intensity (Bradstock *et al.* 2002; Cary *et al.* 2003). Hence, there are potentially important interacting effects between, for example, fire and the addition of CWD. Similarly, biomass removal by grazing animals may influence the intensity and frequency (and effects) of prescribed burning. The experimental design allows estimation of significant interactions.

Statistical design

In both experiments, polygons were paired so that all eight combinations of CWD treatments and fire occur on one site in each pair. Effectively, the CWD treatments were all combinations of two factors, one being with and without 'clumped', and the other being with and without 'dispersed' CWD. The combinations of fire and treatments have a 2 x 2 x 2 structure. Comparisons between polygons are likely to be less accurate than comparisons of sites within polygons, and the treatments were allocated so that no treatment main effects were confounded with differences between polygons. In any of the six polygon pairs, one degree of freedom has to be confounded with an interaction term. The design ensured that the three factor interaction was confounded with polygons in three pairs in each experiment, and each two factor interaction was confounded with polygons in one pair of each experiment. This enables us to estimate the two factor interactions with a high degree of accuracy, while retaining the ability to detect a three factor interaction should it prove to be important. Apart from vegetation structure, all the treatments were allocated at random in accordance with the design.

Thus, our design provides a framework for estimating and assessing the statistical and practical significance of a rich set of hypotheses (enumerated in the ANOVA table, Table 1 and 2). In other words, for each reserve, we can quantify the effects of experimental class, kangaroo exclusion, fire, clumped wood, dispersed wood, and all 2-way and many 3-way interactions between these factors, as well as estimate variability at several levels.

Response variables - rationale

A range of response variables has been chosen to examine the effects of the experimental manipulations on the structure, composition, ecological processes and biodiversity in the reserves. These are: the lower structural component of the vegetation and the abundance and diversity of birds, small mammals, reptiles and invertebrates. The rationale for including these response variables is described below.

Vegetation

Plants are critical to the structure and function of the Yellow Box – Blakely's Red Gum Grassy Woodland ecosystem and are a major component of biodiversity. A key effect of human-disturbance of woodlands has been the simplification of habitat through thinning and removal of woody vegetation, changes in groundlayer composition and prevention of tree and shrub regeneration (Yates and Hobbs 1997; Hobbs and Yates 2000). Vegetation assessments were undertaken for two key purposes: (1) to evaluate the effect of treatments on vegetation structure and composition through time, and (2) to provide covariates for inclusion in analysis of faunal responses.

Birds

The decline in woodland birds associated with the loss and modification of temperate woodlands in Australia is recognized as a serious conservation problem (Robinson and Traill 1996; Garnett and Crowley 2000; Ford *et al.* 2001). Yellow Box-Blakely's Red Gum Grassy Woodlands in the ACT support a range of bird species, including eight species listed as threatened in that Territory (ACT Government 2004a). An additional four species, typical of Yellow Box-Blakely's Red Gum Grassy Woodlands, are listed as vulnerable in neighbouring New South Wales (ACT Government 2004a). All 12 species have been recorded in Mulligans Flat or Goorooyarroo. However, of the resident species, the Hooded Robin *Melanodryas guttata* is very localised, Diamond Firetail *Stagonopleura guttata*, Varied Sitella *Daphoenositta chrysoptera* and Crested Shrike-tit *Falcunculus frontatus* are rarely seen and the Brown Treecreeper *Climacteris picumnus* disappeared in 2000 from Mulligans Flat and in 2005 from Goorooyarroo (J. Bounds personal communication).

Small mammals

Since European settlement, there has been a severe decline in mammalian fauna in temperate woodlands (Burbidge and McKenzie 1989; Dickman 1994; Short and Smith 1994; Short 1998; Burbidge and Manly 2002). This is due to the combined effects of introduced predators, such as foxes *Vulpes vulpes* and cats *Felis catus*, competition

for food from introduced herbivores such as domestic livestock, rabbits *Oryctolagus cuniculus* and hares *Lepus europaeus*, overgrazing by native fauna, habitat loss and modification, and altered fire regimes (Morton 1990; Short and Smith 1994; Smith and Quinn 1996). Small to medium sized mammals (35 g - 5.5 kg) have been particularly affected (Burbidge and McKenzie 1989; James and Eldridge 2007). Many small ground-foraging mammals had important ecological effects such as mixing of organic material into soils, soil aeration, spreading of mycorrhizae and seeds, improved water infiltration and germination of seeds (Claridge *et al.* 1992; Garkaklis *et al.* 1998; 2003; Martin 2003). Like elsewhere in Australia, many small mammals have become rare or locally extinct in the ACT since European settlement (ACT Government 2004a). The effects of woodland manipulations on surviving small mammal populations is of significant conservation interest and is a key starting point for the ecological research. In the future, reintroduction of some locally extinct species into the feral animal-proof fenced reserve at Mulligans Flat will provide added research opportunities (see below).

Two small native mammals of conservation interest have been recorded in the Mulligans Flat – Goorooyarroo reserves or surrounding areas in recent times. These are the Yellow-footed Antechinus *Antechinus flavipes* and the Common Dunnart *Sminthopsis murina* (Frawley 1991). The current status of both species in these reserves is poorly known. This experiment aims to establish whether these species are still present at the experimental sites, and whether manipulations can improve the habitat for these species.

Reptiles

Reptiles are thought to be particularly vulnerable to habitat loss, fragmentation and degradation. This is because they are sedentary, have relatively low mobility and forage and live in ground layer substrates and associated microhabitats that are most likely to be affected by degrading processes (Brown 2001; Brown *et al.* 2008). Reptiles may also be preyed by feral foxes and cats. Consequently, the effect of experimental treatments and the feral animal-proof fence on reptiles is of conservation interest.

Invertebrates

Invertebrates constitute a large proportion of multi-cellular species on Earth (Stork 2007), and have a critical role in ecosystem processes (Samways 2005). However, despite their abundance, diversity and ecological importance, most studies of biodiversity focus on birds, large mammals and plants (Stork 2007). Invertebrates are included in this experiment because of: (1) their abundance, (2) the diversity of functional groups, (3) their role in ecosystem processes, (4) their relatively small scale of operation – which was appropriate for some treatments, (5) their short generation time and associated rapid response to changed environmental conditions, (6) their potentially rapid response to woodland manipulations, and (7) their importance as a food source for other fauna (e.g. *S. murina*; Fox and Archer 1984). Detailed analysis of the beetle data collected in this experiment will be published elsewhere (e.g. Barton *et al.* 2009), and the responses of other invertebrate groups will be analyzed at a later date.

Response variables - measurement

Vegetation measurements

Vegetation surveys were split into two categories:

- (1) Ground layer – below 0.5 m in height and up to 2 cm diameter at breast height (DBH);
- (2) Trees – over 0.5 m in height and over 2 cm DBH.

Ground layer and soil measurements

A survey of the ground layer vegetation and ground cover characteristics was conducted in Spring 2007, and soils in Autumn 2008, with the methods and results presented in detail in McIntyre *et al.* (2010). In summary, the survey was conducted across each of the 96 1 ha sites, using 30 systematically-located quadrats (0.5 x 0.5 m), per site. The top six species (or species groups) were ranked by biomass in each quadrat, and ground cover (litter, litter depth, bare ground, cryptogams, live plant basal area, rock and logs) was measured at four points in each quadrat. Total biomass estimates were made for each quadrat (non-destructive) and these were combined with the ranks to obtain species abundances using the BOTANAL methodology (Tothill *et al.* 1992). Soil samples were taken in Autumn 2008 to a depth of 10 cm at each quadrat location and pooled to obtain an average for each site. The following analyses were performed by the Victorian Department of Primary Industries Laboratory, Werribee Victoria: nitrate-n (mg/kg), total carbon (g/100g), total nitrogen (g/100g), C:N ratio, organic matter (g/100g), electrical conductivity (dS/m), pH(CaCl₂), total soluble salts (%), available phosphorous (Colwell) (mg/kg), texture, colour.

Tree measurements

All trees over 2 cm DBH were measured at each site and a GPS location was recorded for each tree over 10 cm DBH. Tree species and health also were recorded. Area coverage of tree regeneration below 2 cm DBH was measured. Where amounts of regeneration were relatively small, absolute counts were made. The aim of this approach was to record the tree population structure before treatments were implemented. This will provide covariates which can be used to examine the relationship between woodland community, and CWD accumulation, and ultimately to allow process and spatial modelling of past and future scenarios. The detailed results of vegetation baseline surveys will be presented elsewhere.

Bird survey

Birds surveys were undertaken in October and November in 2006 by experienced bird observers. Birds were surveyed for 10 minutes using the point count method, at each star picket at the 50 m and 150 m position (i.e. two per site). Each observer was randomly allocated a polygon containing four sites each morning. Counts took place from 6:30am until completion, and cold, windy or wet conditions were avoided. Birds respond at a range of spatial scales, from the site level through to the landscape level. Therefore, observers noted the presence and abundance of birds in concentric bands (0 – 25 m, 25 – 50 m, 50 – 100 m and over 100 m and overhead). Data

collected up to 50 metres from the observer were used for the analysis in this paper. Observer heterogeneity in bird counts is well known (e.g. Kavanagh and Recher 1983; Recher 1988; Cunningham *et al.* 1999; Lindenmayer *et al.* 2009). To allow for this heterogeneity, two counts were conducted at each point by two different observers, on different days (*sensu* Cunningham *et al.* 1999). Therefore, each site had four point counts each year. The order in which sites were surveyed was reversed on the second day to minimize any effect of time of survey.

Small mammal survey

Pilot surveys with Elliott traps in Goorooyarroo Nature Reserve did not reveal the presence of any small mammals. Footprint tracking tunnels were, therefore, chosen as the best survey technique, in light of the likely low small mammal densities. Tracking tunnels have been used extensively for surveying rodents (King and Edgar 1977; Stokes *et al.* 2004). In this study, tunnels were made from 400 mm of conduit. Inside each tunnel was a plastic plate with an ink pad in the middle, and two 120 mm by 90 mm pieces of paper on either side. The ink used was a mixture of water (800 ml), food dye (200 ml) and poly-ethylene glycol (200 ml) to prevent drying. A pea-sized smear of peanut butter was placed on the side of the tunnel, above the ink pad as an attractant.

During each survey period, four tracking tunnels were placed in each site (2 tunnels, 10 m either side of each star picket along the central axis of the site). Traps were put out on Monday and collected on Friday (16 trap nights per site, 768 per reserve, 1536 in total). Footprints were identified using a reference collection developed by CSIRO Sustainable Ecosystems. Trapping took place in November/ December 2006 and May/June 2007. Extensive periods of rain in December 2007 made trapping impossible for that period.

Reptile survey

In preliminary studies, pitfalls were trialed at a subset of sites in Goorooyarroo (3 x 20 l buckets set 10 m apart and connected by a 20 m drift fence). However, installation of buckets caused considerable soil disturbance and the nature of the local soils conditions meant these (a) were difficult to install; and (b) were pushed out of the ground in wet conditions. Preliminary active searches were trialed at a sub-set of sites within 50 m of each star pickets. A comparison of results derived from the two approaches was made, and it was found that there were no significant differences in species richness or detection of individual species between methods. It was, therefore, decided that active searches would be used for the full experiment.

Active searches were conducted at the end of summer 2007. This was done to detect adults as well as the young from the preceding spring/summer period. Active searches were made at all 96 1 ha sites over a period of 30 minutes each. Observers searched substrates such as logs, rocks and bark, and also scanned the logs for basking reptiles with binoculars. The location and substrate also were noted. As with bird surveys, observers were assigned to polygons randomly, each site was observed by two observers on two different days and sites were surveyed in reverse order on the second day. Amphibians, were not a target of this survey method, but were recorded when found.

Statistical analysis

The variety and complexity of the data being collected requires many different models for their statistical analysis. The packages GenStat (VSN International Ltd, Hemel Hempstead, UK) and R (R Development Core Team 2010) are being used for the bulk of the statistical computation. Statistical methods to be used will include analysis of variance and the fitting of generalized linear mixed models, as well as multivariate methods such as correspondence analysis (Greenacre 2007). The effect of spatial dependence between sites should be largely eliminated by the randomization. However methods outlined in Diggle and Ribeiro (2007) will be also used to explicitly deal with possible residual spatial dependence. This will guard against spuriously inferring significant treatment and covariate effects which might result from ignoring spatial correlation. Where possible, all analyses will be undertaken or supervised by practicing statistical professionals to ensure the latest techniques emerging from statistical science are utilized where appropriate.

Results

Baseline bird surveys 2006

A total of 85 bird species was detected in the 2006 bird surveys (74 in Mulligans Flat and 71 in Goorooyarroo). The total number of individual birds detected within 50 metres of observers was 2370 in Mulligans Flat and 2290 in Goorooyarroo (4660 in total). Of the 12 woodland birds listed as threatened in the ACT, New South Wales or other jurisdictions, five were found to be present in the two reserves. These were the *M. cucullata* (seven observations in Mulligans Flat but zero in Goorooyarroo), White-winged Triller *Lalage sueurii* (24 in Mulligans Flat, 14 in Goorooyarroo), *D. chrysoptera* (10 in Mulligans Flat, 10 in Goorooyarroo), Superb Parrot *Polytelis swainsonii* (one in Mulligans Flat and 37 in Goorooyarroo) and Speckled Warbler *Chthonicola sagittata* (five in Mulligans Flat and two in Goorooyarroo). Two additional species were detected which are also considered of conservation concern or in low numbers despite suitable habitat in the ACT (Cunningham and Rowell 2005). These were the Dusky Woodswallow *Artamus cyanopterus* (two in Mulligans Flat and three in Goorooyarroo) and the Jacky Winter *Microeca fascians* (four in Mulligans Flat; zero in Goorooyarroo).

Species richness was calculated at each site for all birds within 50 m of the observer. Analyses of variance of the logarithm of species richness in 2006 at Goorooyarroo and at Mulligans Flat are shown in Table 1. On average, fourteen species were detected per site. Analysis of baseline bird data showed that within-polygon variation was smaller than between-polygon variation. This means that sites within polygons are more similar to each other than sites from different polygons. Between-site variances were similar for the two reserves, but differences between polygons were greater at Goorooyarroo. In the analyses, 30 variance ratios were calculated, and two of these were significant at the 5% level. This is consistent with what would be expected in the absence of treatment effects.

Table 1 – Table showing analyses of variance of the logarithm of bird species richness for the two reserves in 2006. Species richness calculated from all birds within 50 m of the observer at a site. Interactions of more than two factors are not included.

		Goorooyarroo Nature Reserve		Mulligans Flat Nature Reserve	
Term	Degrees of freedom	Mean square	Significance level	Mean square	Significance level
Polygon pair stratum					
Experimental class (EC)	2	0.252		0.024	
Kangaroo exclusion (KE)	1	0.306		0.327	
EC × KE	2	0.667		0.019	
Polygon stratum					
Burn × Clumped	1	0.015	0.86	0.010	0.79
Burn × Dispersed	1	0.348	0.43	0.024	0.69
Clumped × Dispersed	1	0.001	0.96	0.084	0.47
Residual	3	0.429		0.122	
Site stratum					
Burn	1	0.046	0.33	0.168	0.06
Clumped	1	0.013	0.60	0.071	0.21
Dispersed	1	0.202	0.05	0.000	0.99
Burn × Clumped	1	0.067	0.24	0.146	0.08
Burn × Dispersed	1	0.236	0.03	0.023	0.47
Clumped × Dispersed	1	0.000	0.99	0.005	0.73
Burn × EC	2	0.106	0.12	0.010	0.80
Clumped × EC	2	0.043	0.40	0.078	0.18
Dispersed × EC	2	0.083	0.19	0.001	0.97
Burn × KE	1	0.013	0.60	0.022	0.48
Clumped × KE	1	0.000	0.98	0.003	0.79
Dispersed × KE	1	0.000	0.95	0.002	0.82
Residual	21	0.046		0.042	
Total	47	0.123		0.051	

Baseline active searches for reptiles in 2007

After 2880 minutes of active searches in 2007, 265 individual reptiles were detected (146 in Goorooyarroo and 119 in Mulligans Flat). A total of 12 reptile species was detected in active searches (nine in Mulligans Flat and ten in Goorooyarroo). The two species detected in Mulligans Flat that were not detected in Goorooyarroo were the Spotted-backed Skink *Ctenotus orientalis* and Dwyer's Snake *Suta dwyeri*. The three species detected in Goorooyarroo and not Mulligans Flat were the Eastern Brown Snake *Pseudonaja textilis*, Common Blue Tongue *Tiliqua scincoides* and Garden Skink *Lampropholis guichenoti*. The most commonly detected reptile was Boulenger's Skink *Morethia boulengeri* (71 observations) in Goorooyarroo and Delicate Skink *L. delicata* (37 observations) in Mulligans Flat. In pilot pitfall trapping in Goorooyarroo in 2004, two additional reptile species were detected, the Blind Snake *Ramphotyphlops nigriscens* and the Stone Gecko *Diplodactylus vittatus*.

Results of analyses of variance of the logarithm of species richness+1 in 2007 Mulligan's Flat and Goorooyarroo are shown in Table 2. On average 1.4 species were detected per site. Three of the 30 variance ratios calculated were significant at the 5% level. Again this is consistent with what might be expected in the absence of treatment

effects. In this case, the variation between sites from different polygons was similar to the variation between sites from the same polygon.

In total, 61 individual amphibians were detected in 2007 (one observation in Goorooyarroo and 60 in Mulligans Flat). Three species of amphibian were detected (three in Mulligans Flat and one in Goorooyarroo). Analyses of variance for both abundance and species richness of amphibians did not give meaningful results because of the small numbers of animals involved.

Baseline small mammal tracking tunnels 2006 and 2007

Few small mammals were detected in Mulligans Flat and Goorooyarroo Nature Reserves in both tracking tunnel periods, despite considerable survey effort (3072 trap nights). In the two surveys (2006 and 2007), four mammal species were detected: possum spp. (a non-target species), *S. murina*, House Mouse *Mus musculus* and rat *Rattus* spp. Of the target species, *M. musculus* was detected only in Goorooyarroo in 2006 (two individuals), and *S. murina* in 2007 only (six individuals). Of the target species in Mulligans Flat, two *S. murina*, one *M. musculus* and one *Rattus* spp. were detected in 2006, and only *S. murina* was detected in 2007 (one individual). No *A. flavipes* was detected.

Table 2. Table showing analyses of variance of the logarithm of reptile species richness+1 for the two reserves in 2007. Species richness calculated from all reptiles observed at a site. Interactions of more than two factors are not included.

		Goorooyarroo	Nature Reserve	Mulligans Flat	Nature Reserve
Term	Degrees of freedom	Mean square	Significance level	Mean square	Significance level
Polygon pair stratum					
Experimental class (EC)	2	0.066		0.380	
Kangaroo exclusion (KE)	1	0.123		0.005	
EC x KE	2	0.219		0.149	
Polygon stratum					
Burn x Clumped	1	0.240	0.41	0.004	
Burn x Dispersed	1	0.541	0.24	0.060	
Clumped x Dispersed	1	0.021	0.80	0.000	
Residual	3	0.258		0.000	
Site stratum					
Burn	1	0.111	0.43	0.075	0.59
Clumped	1	0.248	0.24	0.391	0.22
Dispersed	1	0.934	0.03	0.000	0.98
Burn x Clumped	1	0.412	0.13	0.244	0.33
Burn x Dispersed	1	0.563	0.08	0.631	0.13
Clumped x Dispersed	1	0.236	0.25	0.019	0.78
Burn x EC	2	0.069	0.67	0.220	0.43
Clumped x EC	2	0.090	0.60	0.133	0.59
Dispersed x EC	2	0.128	0.48	0.201	0.46
Burn x KE	1	0.018	0.75	1.228	0.04
Clumped x KE	1	0.011	0.94	0.197	0.38
Dispersed x KE	1	1.001	0.02	0.180	0.40
Residual	21	0.168		0.249	
Total	47	0.123		0.051	

In summary, small mammals were detected at very low densities in both reserves, and it was consequently not possible to test for treatment effects. For this reason, it was decided to suspend trapping until all treatments were in place and the feral animal-proof fence was constructed in Mulligans Flat (completed in June 2009).

Ground layer and soil measurements

Several vegetation and soil variables were analysed to check for pre-treatment effects. The number of significant variance ratios calculated was consistent with the assumption of no effects prior to the application of treatments. However, for some variables, between-polygon variation was substantially larger than within-polygon variation. A summary of comparisons for key variables is presented in Table 3.

Total ground layer biomass in both reserves was low, reflecting the ongoing drought and high levels of kangaroo grazing (Mulligans Flat: range 204 to 2352, mean 592, median 381 kg/ha; Goorooyarroo: range 289 to 1833, mean 289, median 430 kg/ha). An unfertilized *Themeda* (kangaroo grass) sward in this region has the production potential 3200 kg ha⁻¹yr⁻¹ in the Southern Tablelands (Keys 1996). The *Themeda* swards in the reserves sampled in spring 2007 had an average biomass (green and dead)

of 352 kg/ha. In addition, it is recommended that after a drought, grasslands need to accumulate 800-1000 kg/ha green matter to give plants time to replenish their energy reserves (Keys 1996).

Discussion

A number of key conclusions emerge from the analysis of baseline data from the Mulligans Flat-Goorooyarroo Woodland Experiment:

1. the lack of pre-treatment effects shows that the experimental framework provides a strong inferential framework for tracking the effects of habitat manipulations;
2. while the number of bird species observed was relatively high, some species of conservation concern were absent or in low numbers;
3. small mammal species that are typical of Yellow Box-Blakely's Red Gum Grassy Woodlands (see Van Dyck and Strahan 2008) are absent or in very low densities;
4. reptile species richness and abundance was relatively low. Large snakes were detected at very low densities;
5. total plant biomass, which underpins the woodland ecosystem, was very low in most sites (see detailed discussion in McIntyre *et al.* 2010.). Low biomass

Table 3. Comparison of between and within polygon residual variation for vegetation and soil variables

Variable	Goorooyarroo Nature Reserve		Mulligans Flat Nature Reserve	
	Between polygons	Within polygons	Between polygons	Within polygons
Total biomass(t/ha)	0.196	0.050	1.07	0.13
Litter %	157.4	42.9	198.3	56.8
Mean litter depth	1.65	4.98	7.86	9.84
NO ₃	9.36	4.06	3.41	1.98
Total carbon %	0.96	0.15	2.24	0.17
Total nitrogen %	0.00185	0.000623	0.00904	0.00076
Organic matter %	3.16	0.52	7.74	0.57
pH CaCl ₂	0.251	0.027	0.102	0.011
Phosphorus	15.5	4.9	41.6	4.52

means that the grassy understorey in much of the reserve area has developed a lawn structure (e.g. Figure 4) which is insufficient to provide habitat for many faunal species (McIntyre 2005) and soil functioning (McIntyre and Tongway 2005).

Overall, baseline faunal surveys suggest that even the good example of Yellow Box-Blakely's Red Gum Grassy Woodland at Mulligans Flat and Goorooyarroo Nature Reserves is in a lesser quality condition for some key faunal groups expected. The lack of genuine significant effects prior to application of treatments shows that effects observed in the future are likely to reflect genuine differences between experimental treatments.

Birds

The number of birds observed and species richness was reasonable, but lower than surveys in similar woodlands conducted at the same time of year (Bounds *et al.* 2010). For example, temperate woodland bird surveys on the south-west slopes of New South Wales have resulted in the detection of 119 (Lindenmayer *et al.* 2008) and 159 (Cunningham *et al.* 2008) bird species respectively. The absence or low densities of threatened bird species that should be typical of these woodlands in Mulligans Flat - Goorooyarroo is a matter for conservation concern. Of the resident threatened species or species of concern that have been recorded in Mulligans Flat or Goorooyarroo in the past (Cunningham and Rowell 2005), three were not detected in surveys. These were *C. picumnus*, *S. guttata*, and *F. frontatus*.

Small mammals

The results of baseline surveys show that small mammals were found at very low densities in Mulligans Flat and Goorooyarroo Nature Reserves. Elliot trapping in Goorooyarroo produced no captures. This agrees with findings of Fischer (unpublished report) who conducted Elliot trapping in Mulligans Flat in 1999 and caught no small mammals in 900 trap nights. With such low small mammal densities, Elliot trapping is not considered a cost-effective trapping technique at this point in time. Footprint tracking tunnels were more successful than Elliot traps –

especially because *S. murina* can be detected with this method. The continued presence of *S. murina* is a significant discovery because the presence of the species was previously uncertain. *Antechinus flavipes* was not detected, and now appears to be absent from the reserves. If *A. flavipes* is still present in the reserves, it is probably occurs very locally and at very low densities. If it is still present, it may naturally increase with removal of feral predators, addition of CWD and exclusion of kangaroos. Additional surveys targeted at specific key habitat elements in Mulligans Flat and Goorooyarroo were undertaken in August 2008 but no *A. flavipes* were detected (Victoria Sheean, unpublished data).

The almost complete absence of *M. musculus* was unexpected. However, this may be because new suburbs have only recently been established on the boundaries of the reserves and are yet to have an effect in terms of elevating numbers of this exotic pest rodent species. It is anticipated that *M. musculus* numbers will increase as suburbs develop and future monitoring should detect any changes.

Reptiles

The species richness of reptiles (12 across both reserves) was lower than some studies in similar habitat. For example, Cunningham *et al.* (2007) detected 22 species in a region west of the ACT using both active searches and artificial substrates. In another study, also west of Canberra, Fischer *et al.* (2004) detected 18 species using pitfalls and active searches. However, species detections were similar to those found by Brown *et al.* (2008) in Victoria (10 species, 152 individuals) using transect censuses and active searches.

Another interesting outcome of our surveys was that large individuals of *P. textilis* were rarely observed (although juveniles were captured in pits, and shed skins were found). The species appears to be at low densities. This may be due to low detection using the active search methodology or the relatively low number of prey as indicated in other surveys. If small mammals, reptiles and amphibian numbers increase in response to treatments, it is predicted that the number of large brown snakes will increase in the future.

The future

Our analysis of baseline data demonstrates that the experimental design provides a strong inferential framework for future research. As well as ongoing monitoring of post-treatment effects, new research opportunities also will be able to take advantage of the experimental design. With the building of the Mulligans Flat Woodland Sanctuary, which encloses the Mulligans Flat half of the experiment with a feral animal-proof fence (Figure 2), there will be considerable scope for further research, such as on the interaction of current experimental treatments, the absence of feral foxes, cats, rabbits and hares, and the reintroduction of locally extinct species. Such restoration experiments, under

controlled conditions and with active collaboration between researchers and the managers of the nature reserve, provide unique opportunities for research that might not otherwise be possible. These conditions will allow the experimental investigation of the ecological processes associated with reconstructing Yellow Box-Blakely's Red Gum Grassy Woodland.

While post-treatment responses to the experimental treatments are anticipated within the lifetime of the current funding up to (December 2010), the effects of treatments will continue to develop over the long-term. It is therefore planned that this experiment will be a long-term ecological research site for understanding temperate woodland restoration.

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